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Abstract: **BACKGROUND:** Patients sensitized to birch pollen frequently suffer from a food allergy to plant foods such as celery, carrots, or hazelnut. One of the main manifestations of birch pollen-related food allergy is the oral allergy syndrome. Skin tests and allergen-specific immunoglobulin (Ig) E determinations are poor predictors of such reactions when assessed by double-blind placebo-controlled food challenge (DBPCFC). **OBJECTIVE:** To investigate whether a cellular test based on leukotriene release from basophils, the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA), is predictive of pollen-related food allergy. **METHODS:** Birch pollen-sensitized patients with positive DBPCFC to celery (n=21), hazelnut (n=15), and carrot (n=7) underwent skin tests along with determination of specific IgE and CAST-ELISA for the respective allergens. The results were compared with those of 24 birch pollen-sensitized patients with negative open food challenge to celery, hazelnut, and carrot. **RESULTS:** While skin prick tests had a sensitivity of 85%, 80%, and 29% for commercial extracts of celery, hazelnut, and carrot, respectively, prick testing with self-prepared extracts yielded sensitivities of 100%, 80%, and 100%, respectively. For specific IgE determinations, sensitivities were 71%, 73%, and 57%, respectively, and the respective specificities were 67%, 73%, and 60%. For CAST-ELISA with various sources and doses of allergens, the sensitivity varied from 71% to 95% for celery, 73% to 80% for hazelnut, and 43% to 86% for carrot. The respective specificities were 67% to 92%, 75% to 88%, and 77% to 91%. Analysis of the predictive value of CAST-ELISA with receiver operating characteristic curves showed that the results of the tests were more predictive of pollen-related food allergy than quantitative allergen-specific IgE determinations. **CONCLUSIONS:** CAST-ELISA is more specific than routine diagnostic tests for the diagnosis of pollen-related food allergy to celery, hazelnut, and carrot.

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Predictive Value of the Sulfidoleukotriene Release Assay in Oral Allergy Syndrome to Celery, Hazelnut, and Carrot

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Sulfidoleukotriene Release Assay in Oral Allergy Syndrome

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Abstract

Background: Patients sensitized to birch pollen frequently suffer from a food allergy to plant foods such as celery, carrots, or hazelnut. One of the main manifestations of birch pollen-related food allergy is the oral allergy syndrome. Skin tests and allergen-specific immunoglobulin (Ig) E determinations are poor predictors of such reactions when assessed by double blind placebo-controlled food challenge (DBPCFC).

Objective: To investigate whether a cellular test based on leukotriene release from basophils, the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA), is predictive of pollen-related food allergy.

Methods: Birch pollen-sensitized patients with positive DBPCFC to celery (n=21), hazelnut (n=15), and carrot (n=7) underwent skin tests, specific IgE determinations, and CAST-ELISA to the respective allergens. The results were compared with those of 24 birch pollen-sensitized patients with negative open food challenge to celery, hazelnut, and carrot.

Results: While skin prick tests had a sensitivity of 85%, 80%, and 29% for commercial extracts of celery, hazelnut, and carrot, respectively, prick testing with self-prepared extracts yielded sensitivities of 100%, 80%, and 100%, respectively. For specific IgE determinations, sensitivities were 71%, 73%, and 57%, respectively, and the respective specificities were 67%, 73%, and 60%. For CAST-ELISA with various sources and doses of allergens, the sensitivity varied from 71% to 95% for celery, 73% to 80% for hazelnut, and 43% to 86% for carrot. The respective specificities were 67% to 92%, 75% to 88%, and 77% to 91%. Analysis of the predictive value of CAST-ELISA with receiver operating characteristic curves showed that the results of the tests were more predictive of pollen-related food allergy than quantitative allergen-specific IgE determinations.

Conclusions: CAST-ELISA is more specific for the diagnosis of pollen-related food allergy to celery, hazelnut and carrot than routine diagnostic tests.

Key words: Cellular antigen stimulation test. Sulfidoleukotriene release. Pollen-associated food allergy. Oral allergy syndrome. Double-blind placebo-controlled food challenge. Hazelnut. Celery. Carrot.

Introduction

Allergy to foods affects about 3% to 4% of the adult population [1]. One of its most frequent manifestations is the oral allergy syndrome (OAS), characterized by oral and pharyngeal mucosal symptoms following the ingestion of allergenic foods [2,3]. In particular, patients sensitized to birch pollen may develop oral symptoms after ingestion of apples, pears, hazelnuts, kiwi, carrots, celery, and many others [4,5].

Diagnosis of food allergy is based first on the patient's history, followed by skin prick tests (SPT) and analysis of food-specific immunoglobulin (Ig) E. Some of these tests have a relatively high sensitivity but they do not distinguish between pure sensitization and clinically relevant allergy. Thus, many test-positive patients tolerate the corresponding food without problems [6]. For that reason, the gold standard in food allergy continues to be double-blind placebo-controlled food challenge (DBPCFC) [7]. Unfortunately, DBPCFC is a tedious and time-consuming procedure that is not without risk for the patient. Consequently, there is a consensus that any in vitro diagnostic test that could replace or reduce the need for DBPCFC would be of considerable benefit in the diagnosis of food allergy. Cellular tests represent candidates to replace DBPCFC, since the stimulation of IgE-loaded basophils in vitro is, in principle, more representative of the pathophysiologic process occurring in vivo than simple determination of serum IgE concentrations. Since 1993, a new sulfidoleukotriene-release test, the cellular antigen stimulation test (CAST) in combination with enzyme-linked immunosorbent assay (ELISA), known as CAST-ELISA has been shown to be of interest in the diagnosis of IgE-mediated allergies to inhalant allergens, insect venoms, latex, and some drugs [8]. It has also been shown to display high sensitivity and specificity in IgE-mediated allergies to a number of allergenic foods [9,10].

The aim of this study was to investigate whether CAST-ELISA is useful in diagnosis of allergy to hazelnut, celery, or carrot in patients sensitized to birch pollen with or without OAS to these food allergens. In particular, we sought to establish whether CAST-ELISA exhibited predictive value and whether the results of the test were correlated with those of DBPCFC in such patients.

Materials and Methods

Patients

The study included 64 patients aged 16 to 62 years (23 male and 41 female) who were sensitized to birch pollen. All of them had positive SPT results to birch pollen extract and 96.4% also a positive specific IgE test to t3 (birch) allergen, as measured by fluorescent enzyme immunoassay (CAP-FEIA). Twenty-one patients had a positive DBPCFC to celery, 15 to hazelnut, and 7 to carrot. In comparison, 24 birch-sensitized patients had a negative open food challenge to celery, 16 to hazelnut, and 21 to carrots.

Ethical Considerations

The study was reviewed and approved by the Local Ethics Committee of the University of Zurich. All subjects provided signed informed consent before enrollment in the study.

Food Challenges

Food challenges were performed and evaluated as described by Ballmer-Weber et al. [4,5,11]. Birch pollen-allergic patients without a history of a pollen-related food allergy underwent open food challenges to confirm clinical tolerance. To exclude food allergy, those patients ingested 20 g of raw carrots, 20 g of raw celery, and 6 g of raw hazelnuts, respectively.

Kommentar [IKP1]: Do you mean celery? Celeriac is something different; celery is OK!

Skin Testing

SPT was performed with commercial extracts of birch pollen (Soluprick, ALK, Horsholm, Denmark), celery (Allergopharma, Reinbek, Germany), hazelnut, and carrot (Stallergènes, Antony Cedex, France) on the flexor aspect of each patient's forearm with a standardized prick needle (Stallerpoint, Stallergènes). Histamine dihydrochloride (10 mg/mL) was used as a positive control, and the glycerol-containing diluent of the prick solution (Soluprick, ALK-Abello, Horsholm, Denmark) was used as a negative control. Patients also underwent skin testing with self-prepared food extracts, as described previously [4,5,11,12]. Reactions were recorded after 15 minutes. Wheals greater than 3 mm in diameter were considered positive. Skin tests were only done with the suspected culprit food allergen in DBPCFC-positive patients.

In Vitro Testing

Analysis of allergen-specific IgE to birch, celery, hazelnut, and carrots was performed by CAP-FEIA (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. IgE concentrations above 0.35 kIU/L were considered positive.

CAST-ELISA consists of an initial step involving in vitro incubation of isolated blood leukocytes with the putative allergen, in the presence of a low concentration of interleukin 3, for 40 minutes. Then, the concentration of sulfidoleukotrienes (leukotrienes C4, D4, and E4) released are analyzed by ELISA with a highly specific monoclonal antibody [8]. The results are evaluated in terms of the concentration of sulfidoleukotrienes (pg/mL). The test was performed according to the manufacturer's instructions (Bühlmann Laboratories AG, Allschwil, Switzerland) and further details are described elsewhere [8]. The celery, hazelnut, and carrot allergens used for CAST-ELISA were provided by Bühlmann Laboratories and used at final concentrations of 0.8, 4, and 20 ng/mL, respectively. The self-prepared extracts were used at final concentrations of 4, 20, and 100 ng/mL, respectively. To determine the diagnostic sensitivity and specificity of CAST-ELISA with the corresponding food allergens, 20 apparently healthy randomly selected blood donors

with no history of allergy were included. CAST-ELISA reactions to birch pollen allergens were analyzed in 7 patients only. All 7 subjects showed specific stimulations of at least 1615 pg/mL sulfidoleukotrienes.

Statistical Analysis

Thresholds for optimal sensitivity and specificity of CAST-ELISA were established using receiver operating characteristic (ROC) curves according to Zweig and Campbell [13]. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated according to the method of Goldman [14]. The Mann-Whitney test [15] was used to compare CAST-ELISA results between healthy controls, birch pollen-allergic patients without OAS, and DBPCFC-positive patients. A *P* value of less than .05 was regarded as statistically significant.

Results

ROC plots were prepared to determine the cutoff for positivity in CAST-ELISA to achieve optimal sensitivity and specificity in patients with a positive DBPCFC. The results for the different allergen preparations and concentrations are summarized in Table 1. The sensitivities obtained with 0.8 and 4 ng/mL final allergen concentration were too low, and therefore, these data were not considered further. For allergen concentrations between 20 and 100 ng/mL, the optimal cutoff ranged between 50 and 200 pg/mL sulfidoleukotrienes. For the sake of simplicity and direct comparison of individual results, a single cutoff of 120 pg/mL sulfidoleukotrienes was chosen.

The results of comparisons between birch pollen-sensitized patients with either a positive DBPCFC or a negative open food challenge to the ingestion of celery, hazel, or carrot are shown in Table 2. In general, the results obtained with CAST-ELISA using a concentration of 100 ng/mL were less specific (more DBPCFC-negative patients who were CAST-ELISA positive).

With 20 ng/mL of the self-prepared extracts, CAST-ELISA for celery was positive in 85.7% of the DBPCFC-positive patients but only in 8.3% of the challenge-negative subjects. This contrasts with the results of the specific IgE determination, since 33.3% of the challenge-negative subjects were positive. For skin tests, the respective sensitivities were 85% (SPT with commercial extracts) and 100% (SPT with self-prepared extracts). As skin-test data were only available for DBPCFC-positive patients, specificities for SPT were not assessed.

CAST-ELISA using 20 ng/mL of commercial allergen extract for hazelnut was positive in 73.3% of DBPCFC-positive patients and 12.5% of challenge-negative subjects. Analysis of specific IgE was positive in 73.3% and 27.3% of subjects, respectively. A somewhat higher sensitivity of 80% was obtained with skin tests independent of the allergenic extract used.

For carrot, positive CAST-ELISA results were obtained in 85.7% of the DBPCFC-positive patients and in 9.1% of the challenge-negative patients. Analysis of specific IgE was positive in 57.1% and 40%, respectively. SPT with the self-prepared extracts gave a sensitivity of 100%, whereas SPT with the commercial extract showed only 28.6% positivity, suggesting that the commercial carrot extract was not very effective.

For all three food allergens, the positive predictive values and negative predictive values appeared higher for CAST-ELISA than for specific IgE determinations (Table 2).

When tested in normal healthy controls, CAST-ELISA showed 95% to 100% specificity for all 3 food allergens up to a final concentration of 100 ng/mL, independently of the allergen source. However, there were significant differences between allergen sources and concentrations to be used in CAST-ELISA when tested in symptomatic patients (Table 2). The same phenomenon was also observed for the skin tests. The most appropriate allergens to be applied for CAST-ELISA seemed to be the commercial ones (except celery) at a final concentration of 20 ng/mL. The discriminative power of CAST-ELISA under those conditions is shown in Figure 1, where single CAST-ELISA results are shown for healthy controls stimulated with each of the 3 food allergens, challenge-negative patients, most of them stimulated with each of the 3 food allergens, and DBPCFC-positive patients stimulated with the culprit food allergen. The mean net stimulation was 15 pg/mL sulfidoleukotrienes for control subjects, 131 pg/mL for challenge-negative patients, and 1078 pg/mL for DBPCFC-positive patients. Significant differences were observed between the groups ($P < .001$).

The capacity of CAST-ELISA to discriminate between DBPCFC-positive and challenge-negative patients is shown in Figure 2 for the individual food allergens at 20

ng/mL and in Figure 3, where all DBPCFC challenges are considered together and compared to the alternative test methods.

Considering all tested individuals case by case, SPT and analysis of specific IgE were frequently positive in patients without OAS, whereas CAST-ELISA seemed to coincide with clinical reactivity in most cases.

It was recently claimed that quantitative determination of specific IgE has some predictive value in terms of the outcome of DBPCFC [16,17]. The correlation between the results of analysis of specific IgE and DBPCFC to birch and foods (celery, hazelnut and carrot) in our cases is shown in Figure 4. Although a weak correlation was observed for the food allergens, it was clearly weaker than the correlations observed for the qualitative (positive/negative CAST-ELISA) or quantitative measures of CAST-ELISA with DBPCFC.

Discussion

Since its introduction around 1993, CAST-ELISA has been used relatively infrequently in food allergy [8-10,18-19], despite its sensitivity (81%-85%) and specificity (95%-100%) having been reported to be quite high, often higher than that of SPT and/or analysis of specific IgE [9-10,18-19]. Specificities, however, have mostly been determined against a panel of healthy controls, and both clinically validated studies [9,18] have not addressed whether CAST-ELISA discriminates between immunologically sensitized patients—based on SPT results or analysis of specific IgE—who have a positive result in food challenge and those in whom food challenge is negative.

The results of this study show that, in the majority of cases, CAST-ELISA with celery, hazelnut, and carrot allergens discriminates between birch pollen-sensitized patients who show positive or negative results in challenges with these allergenic foods. The greatest discriminatory power lies within a limited range of allergen concentrations. If the concentration is too low (less than 20 ng/mL) the test is not sufficiently sensitive, whereas if the concentration is too high (greater than 100 ng/mL), a positive result may be obtained in some birch-sensitized patients in whom the results of food challenge are negative, even though the results of CAST-ELISA are still negative in healthy nonsensitized controls. Accordingly, the optimal allergen concentration to discriminate prospectively between challenge-positive and challenge-negative patients appears to be around 20 ng/mL of total protein for celery, hazelnut and carrot, and this concentration may also be valid for other allergenic foods. Apart from the optimal allergen concentration, the quality of the allergen extract seems to be crucial and has to be thoroughly controlled for each new batch, as is also true of the commercial CAST allergens. It is apparent, however, that once the appropriate allergen concentration has been determined, CAST-ELISA correlates better with clinical reactivity than SPT or determination of specific IgE concentration.

All currently used test methods are influenced by the phenomenon of immunological cross-reactivity between birch pollen allergens and the related food allergens, and as a result, patients with a positive SPT to the cross-reactive foods may not be clinically allergic to those foods. Thus, it is not surprising that a test based on basophil reactivity might correlate better with clinical reactivity than does determination of IgE concentrations. In addition to the presence of IgE antibodies, this test also takes into account the individual cellular reactivity of each patient [8]. Recently, it has been shown in a similar situation of birch pollen-sensitized patients with plant-related allergy that the flow cytometric basophil activation test can discriminate between patients with and without food allergy [20,21], with a sensitivity of 88% and a specificity of 75% [21].

Further confirmation of the discriminatory power of CAST-ELISA should be obtained through multicenter studies including additional allergenic foods and more severe manifestations of IgE-mediated allergy, such as generalized urticaria, respiratory symptoms, gastrointestinal symptoms, or even anaphylactic reactions. SPT and specific IgE determinations may continue to be first-line diagnostic tools in food allergy, and DBPCFC the gold standard, but CAST-ELISA offers significant advantages as a complementary test that will remove the need for DBPCFC, at least in part, in the routine management of food-allergic patients.

Kommentar [IKP2]: Author:
Please indicate references to such
studies See refs. and new
formulation!

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We are grateful to Prof Alain de Weck for his expert help in preparing and critically reviewing this manuscript. We also thank Marie-Eve Ueberschlag for her expert technical work in evaluating the sulfidoleukotriene release data for the control subjects. We thank Irène Cuhat, Marie-Claire Weber, and Susan Marti for technical assistance and the nurses of the Allergy Unit for their cooperation. Finally, we are grateful to Dr Dirk Lüttkopf of the Paul-Ehrlich-Institute for preparation and characterization of allergen extracts.

Kommentar [IKP3]: Author: Is Prof de Weck aware that he is acknowledged here? Since he was responsible for accepting the manuscript, this is surprising. Yes, Prof. de Weck is aware of being acknowledged here. He helped in preparing this manuscript and submitted it to Prof. Sanz for acceptance of publication in this Journal!

Kommentar [IKP4]: Please indicate which allergy unit

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Legends

Figure 1. Results of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA) in healthy controls (HC), birch pollen-allergic patients without oral allergy syndrome ($t3^+/OAS^-$) and birch pollen-allergic patients with a positive response to double-blind placebo-controlled food challenge (OAS^+). Horizontal bars indicate mean values.

Figure 2. Sensitivity and specificity of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA) for the individual food allergens using double-blind placebo-controlled food challenge (DBPCFC) as the gold standard. CAST-ELISA was performed with the commercial allergens f17 (hazelnut) and f31 (carrot) and with a self-prepared extract of raw celery (f85), all at a concentration of 20 ng/mL. Open columns indicate DBPCFC-positive patients (sensitivity); closed columns, birch pollen-allergic patients without oral allergy syndrome (specificity).

Figure 3. Overall sensitivity and specificity (celery, hazelnut, and carrot) of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA), analysis of specific immunoglobulin E by fluorescent enzyme immunoassay (CAP-FEIA) and skin prick tests (SPT) with commercial (SPT-1) and self-prepared (SPT-2) extracts using double-blind placebo-controlled food challenge (DBPCFC) as the gold standard. OAS^+ indicates patients with oral allergy syndrome, determined by DBPCFC; $t3^+/OAS^-$, birch pollen-allergic patients without OAS ; BL-20, commercial allergens (Bühlmann) at final concentrations of 20 ng/mL; PEI-100, allergen extracts prepared by the Paul Ehrlich Institute at final concentrations of 100 ng/mL.

Figure 4. Correlation between the percentage of patients with a positive double-blind placebo-controlled food challenge (DBPCFC) and the results of the cellular antigen stimulation test in combination with enzyme-linked immunosorbent assay (CAST-ELISA) and fluorescent enzyme immunoassay (CAP-FEIA)..

Table 1. Overall Diagnostic Sensitivity of the Cellular Antigen Stimulation Test with Enzyme-Linked Immunosorbent Assay (CAST-ELISA) According to Cutoff and Allergen Source^a

Allergen Source	Allergen Concentration	Cutoff, pg /mL Sulfidoleukotrienes	Sensitivity, %	
			OAS ⁺	Healthy Controls
Bühlmann (commercial)	20 ng/mL	50	83.7	93.3
		100	74.4	96.7
		120	74.4	98.3
		150	72.1	100
		200	69.8	100
Self-prepared extracts	20 ng/mL	50	79.1	98.3
		100	74.4	100
		120	72.1	100
		150	69.8	100
		200	65.1	100
Self-prepared extracts	100 ng/mL	50	95.3	85.0
		100	90.7	93.3
		120	88.4	96.7
		150	86.0	98.3
		200	86.0	100

Abbreviations: OAS, oral allergy syndrome.

^aThe sensitivity of the cellular antigen stimulation test with enzyme-linked immunosorbent assay was assessed in 20 healthy controls with different sources and concentrations of celery, carrot, and hazelnut extracts, whereas 39 patients with oral allergy syndrome shown by positive double-blind placebo-controlled food challenge were tested with the extract of the culprit foods alone. The data for the 3 different food allergen were taken together and the respective sensitivities and specificities were calculated using various cutoff thresholds.

Kommentar [IKP5]: Author: What do you mean by "summarized"? Also, specificities are not shown in the table. See new formulation!

Table 2. Sensitivity, Specificity, and Positive and Negative Predictive Value for the Different Diagnostic Tests^a

DBPCFC	Test	Allergen Source	Allergen Concentration	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Celery	CAST-ELISA	Commercial	20 ng/mL	71.4	91.7	88.2	78.6
		Self-prepared	20 ng/mL	85.7	91.7	90.0	88.0
		Self-prepared	100 ng/mL	95.2	66.7	71.4	94.1
	CAP-FEIA			71.4	66.7	75.0	62.5
		SPT-1	Allergopharma	44 µg/mL	85.0	nd	na
		SPT-2	Self-prepared	1 mg/mL	100	nd	na
Hazelnut	CAST-ELISA	Commercial	20 ng/mL	73.3	87.5	84.6	77.8
		Self-prepared	20 ng/mL	66.7	87.5	83.3	73.7
		Self-prepared	100 ng/mL	80.0	75.0	75.0	80.0
	CAP-FEIA			73.3	72.7	78.6	66.7
		SPT-1	Stallergènes	6.26 mg/mL	80.0	na	na
		SPT-2	Self-prepared	1 mg/mL	80.0	nd	na
Carrot	CAST-ELISA	Commercial	20 ng/mL	85.7	90.9	75.0	95.2
		Self-prepared	20 ng/mL	42.9	86.4	50.0	82.6
		Self-prepared	100 ng/mL	85.7	77.3	54.5	94.4
	CAP-FEIA			57.1	60.0	40.0	75.0
		SPT-1	Stallergènes	28.6	nd	na	na
		SPT-2	Self-prepared	1 mg/mL	100	nd	na

Abbreviations: CAP-FEIA, fluorescent enzyme immunoassay; CAST-ELISA, cellular antigen stimulation test with enzyme-linked immunosorbent assay; DBPCFC, double-blind placebo-controlled food challenge; na, not analyzed; nd, not determined; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test.

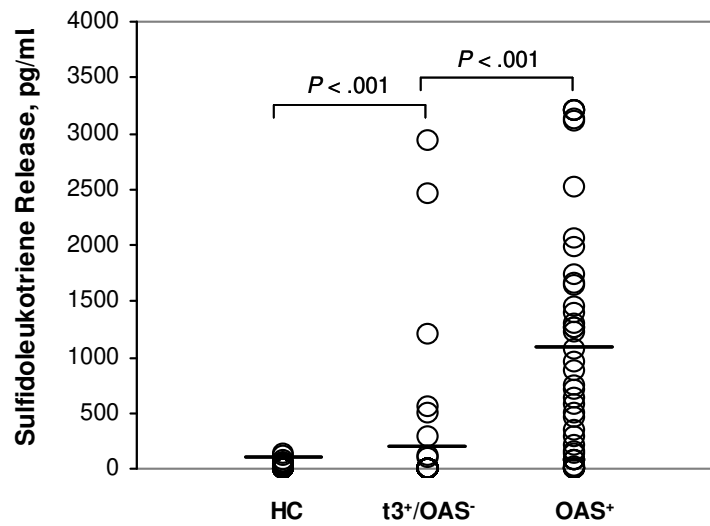
^aTests were performed with commercial food extracts and self-prepared food extracts in patients with positive and negative food challenges to hazelnut, carrot, and celery. DBPFC was used as the gold standard.

Table 3. Individual results of CAST[®]-ELISA, specific IgE (as determined by CAP-*FEIA*), skin prick test with commercial (*SPT-1*) and self prepared (*SPT-2*) extracts in patients with positive and negative food challenge tests. hazelnut (ha, f17); carrot (ca, f31); celery (ce, f85); *OAS*, oral allergy syndrome; *sens*, Sensitization to birch pollen (splgE positive); *SIT*, specific immunotherapy; *w/o*, without; *nd*, not determined.

Kommentar [IKP6]: Author: Provision of raw data in this way seems an inappropriate repetition of information that has already been provided. It should be removed, unless you can explain what relevant additional information it provides. Ok, Table 3 shall be removed!

Patient	Clinical Status	Challenge positive to	CAST-ELISA (pg/ml)			CAP- <i>FEIA</i> (kIU/L)			SPT-1	SPT-2	SPT-1	SPT-2	SPT-1	SPT-2
			f17	f31	ce	f17	f31	f85	ha	ha	ca	ca	ce	ce
1	Birch sens. with OAS	ha	945			1.29			+	++				
2	Birch sens. with OAS	ha	1633			<0.35			-	+				
3	Birch sens. with OAS	ha	503			<0.35			++	++				
4	Birch sens. with OAS	ha	1393			2.88			+++	+				
5	Birch sens. with OAS	ha	<50			2.16			++	+				
6	Birch sens. with OAS	ha	<50			3.44			+	+				
7	Birch sens. with OAS	ha	1218			1.73			+	+				
8	Birch sens. with OAS	ha	<50			4.89			-	+				
9	Birch sens. with OAS	ha	340			0.99			+	-				
10	Birch sens. with OAS	ha	2053			<0.35			+	+				
11	Birch sens. with OAS	ha	1449			0.80			+	++				
12	Birch sens. with OAS	ha	711			<0.35			+	+				
13	Birch sens. with OAS	ca		<50			10.3				+++	++		
14	Birch sens. with OAS	ca		1257			<0.35			-		+++		
15	Birch sens. with OAS	ca		277			5.13			-		+++		
16	Birch sens. with OAS	ca		1261			<0.35			-		+		
17	Birch sens. with OAS	ca		203			0.92			-		++		
18	Birch sens. with OAS	ca		159			<0.35			-		+		
19	Birch sens. with OAS	ce			2466			<0.35					+	++
20	Birch sens. with OAS	ce			449			1.21					+	+
21	Birch sens. with OAS	ce			1787			<0.35					++	+++
22	Birch sens. with OAS	ce			985			<0.35					++	++
23	Birch sens. with OAS	ce			2842			<0.35					+	+++
24	Birch sens. with OAS	ce		<50			0.36						+	+
25	Birch sens. with OAS	ce			1669			2.28					+++	+++
26	Birch sens. with OAS	ce			846			38.3					+++	++
27	Birch sens. with OAS	ce			132			4.77					++	+++
28	Birch sens. with OAS	ce			1917			8.79					+++	+++
29	Birch sens. with OAS	ce			1652			20.6					+++	++
30	Birch sens. with OAS	ce			2549			2.46					+	+
31	Birch sens. with OAS	ce			>3200			6.3					+++	+
32	Birch sens. with OAS	ce			589			0.46					-	++
33	Birch sens. with OAS	ce			1444			<0.35					+++	++
34	Birch sens. with OAS	ce		<50			1.06						-	+
35	Birch sens. with OAS	ce		<50			<0.35						-	+
36	Birch sens. with OAS	ha,ce	3100		>3200	0.61		1.96	+	-			+++	+
37	Birch sens. with OAS	ha,ce	3115		3073	2.78		4.55	-	-			+++	++
38	Birch sens. with OAS	ha,ce	<50		738	0.73		2.41	+	+			++	+++
39	Birch sens. with OAS	ca,ce		>3200	>3200		30.2	0.79			++	+++	++	+++
40	Birch sens. w/o OAS	neg	<50	<50	<50	<0.35	2.41	0.89						
41	Birch sens. w/o OAS	neg	<50	<50	<50	<0.35	<0.35	<0.35						
42	Birch sens. w/o OAS	neg	<50	498	1069	<0.35	0.41	<0.35						
43	Birch sens. w/o OAS	neg	<50	<50	<50	<0.35	<0.35	<0.35						
44	Birch sens. w/o OAS	neg	nd	<50	<50	nd	1.68	1.16						
45	Birch sens. w/o OAS	neg	nd	<50	<50	nd	<0.35	<0.35						
46	Birch sens. w/o OAS	neg	<50	<50	<50	<0.35	<0.35	<0.35						
47	Birch sens. w/o OAS	neg	292	543	<50	0.98	<0.35	<0.35						
48	Birch sens. w/o OAS	neg	<50	<50	137	2.11	1.05	0.65						
49	Birch sens. w/o OAS	neg	nd	nd	<50	nd	nd	0.78						
50	Birch sens. w/o OAS	neg	nd	<50	<50	nd	<0.35	<0.35						
51	Birch sens. w/o OAS	neg	<50	<50	<50	0.65	<0.35	<0.35						
52	Birch sens. w/o OAS	neg	<50	<50	<50	<0.35	<0.35	<0.35						
53	Birch sens. w/o OAS under SIT	neg	<50	<50	<50	<0.35	<0.35	<0.35						
54	Birch sens. w/o OAS under SIT	neg	nd	nd	<50	nd	nd	nd						
55	Birch sens. w/o OAS under SIT	neg	<50	<50	<50	nd	nd	nd						
56	Birch sens. w/o OAS under SIT	neg	<50	<50	<50	nd	nd	nd						
57	Birch sens. w/o OAS under SIT	neg	nd	<50	<50	nd	1.10	1.51						
58	Birch sens. w/o OAS under SIT	neg	nd	<50	<50	nd	nd	nd						
59	Birch sens. w/o OAS under SIT	neg	nd	<50	<50	nd	nd	nd						
60	Birch sens. w/o OAS under SIT	neg	<50	<50	<50	nd	nd	nd						
61	Birch sens. w/o OAS under SIT	neg	<50	<50	<50	nd	nd	nd						
62	Birch sens. w/o OAS under SIT	neg	nd	nd	<50	nd	nd	nd						
63	Birch sens. w/o OAS under SIT	neg	<50	<50	nd	<0.35	0.79	nd						
64	Birch sens. w/o OAS under SIT	neg	2448	<50	<50	nd	nd	nd						

Figure 1



Kommentar [IKP7]: Author:
Please make the following changes
in the figure:
1) Change "sLT release (pg/mL)" to
"Sulfidoleukotriene Release, pg/mL"
2) Change "p<0.0001" to " $P < .001$ "
Done!

Figure 2

Kommentar [IKP8]: Author:
Please change "Sensitivity /
Specificity (%)" to "
Sensitivity/Specificity, %" Done!

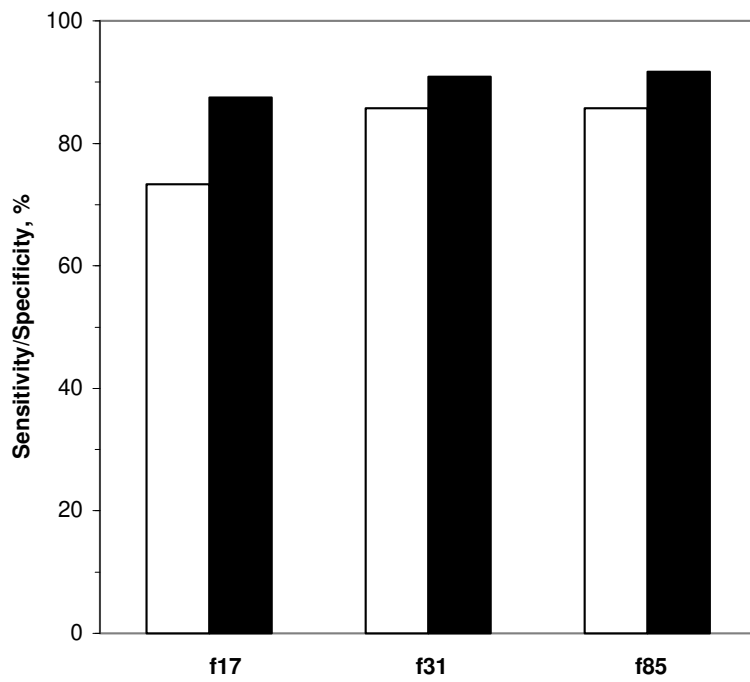


Figure 3

Kommentar [IKP9]: Author:
Please make the following changes
in the figure:
1) Change "CAST" to "CAST-
ELISA" Done!

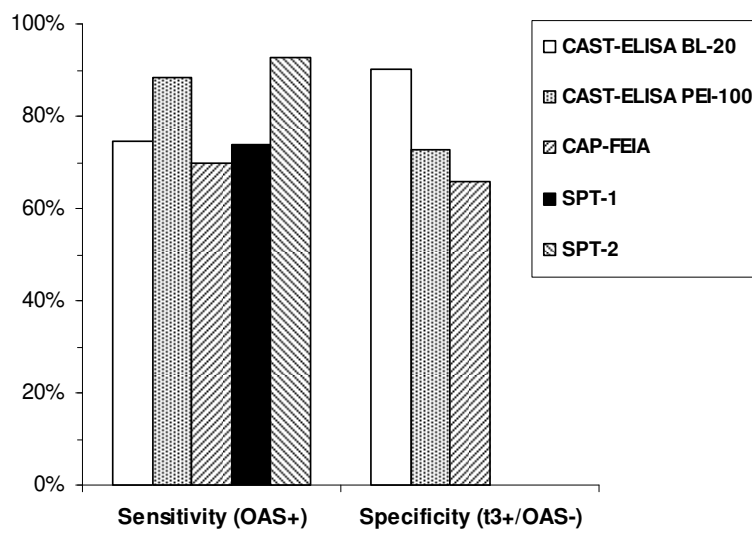
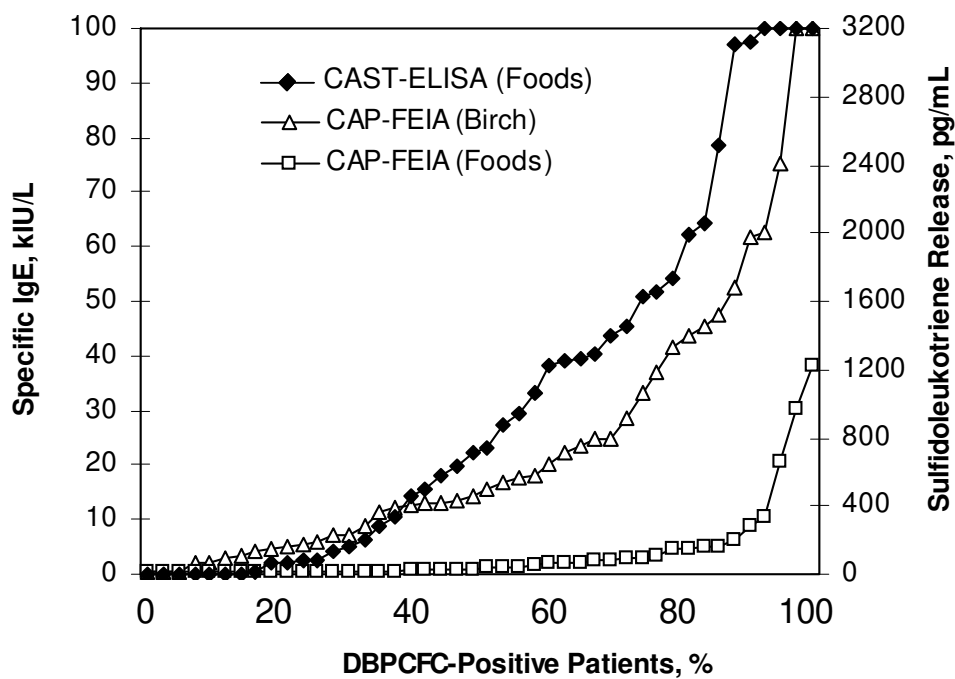


Figure 4



Kommentar [IKP10]: Author:
Please make the following changes
in the figure:
1) Change "spIgE (kIU/L)" to
"Specific IgE, kIU/L"
2) Change "DBPCFC positive
patients (%)" to "DBPCFC-Positive
Patients, %"
3) Change "sLT release (pg/mL)" to
"Sulfidoleukotriene Release,
pg/mL" and change the direction of
the text to face in to the graph
(reading from top to bottom) Done!